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14. ABSTRACT The overall goal of the project was to develop an animal model of the focal dystonia benign essential blepharospasm. Consistent with the widely held view that dystonia results from an interaction between a predisposing condition and an environmental trigger, we proposed to use 7 Hz deep brain stimulation of the basal ganglia as the predisposing condition and dry eye as an environmental trigger to model blepharospasm in rodents. This reporting year we demonstrated that 7 Hz is a critical frequency for basal ganglia activity to create the predisposing condition for the development of blepharospasm. In addition, we showed that basal ganglia modulation of the superior colliculus can produce the exaggerated trigeminal excitability typical of blepharospasm. Dystonia, benign essential blepharospasm, dry eye, motor plasticity, basal ganglia, deep brain stimulation, eyelids, blinking					
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1. Introduction

The focal dystonia benign essential blepharospasm (**BEB**), arises from the convergence of a predisposing condition and an environmental trigger (Hallett et al., 2008). The overall goal of our project is to develop an animal model of BEB based on the hypothesis that hypersynchronized, 7 Hz neuronal oscillations of the basal ganglia create the predisposing condition and that eye irritation from dry eye is the environmental trigger. Our demonstration that hypersynchronized oscillations in the basal ganglia produced by 7 Hz deep brain stimulation of the subthalamic nucleus (**STN DBS**) exaggerate neural plasticity in normal rats (Kaminer et al., 2014) provides a neural mechanism by which hypersynchronized basal ganglia activity creates a predisposing condition. From our studies showing that dry eye initiates neural plasticity in blink circuits to produce compensatory modifications in blinking (Evinger et al., 2002; Schicatano et al., 2002), we predict that combining 7 Hz STN DBS and dry eye would exaggerate neural plasticity and force the normally compensatory adaptive processes in response to dry eye to transform into the characteristics of BEB, *e.g.*, spasms of lid closure, excessive blinking, and trigeminal hyperexcitability. We propose two Specific Aims to test this hypothesis. The goal of the first Specific Aim is to show that synchronized theta oscillations in the basal ganglia exaggerate plasticity in the cerebellum and the excitability of trigeminal blink circuits as occurs in BEB patients. The Major Tasks to accomplish Specific Aim 1 are: 1) to investigate effects of synchronized basal ganglia oscillations on activity of the deep cerebellar nucleus neurons; and 2) to investigate the effects of synchronized basal ganglia oscillations on the activity of superior colliculus neurons. The purpose of the second Specific Aim is to demonstrate that synchronized 7 Hz oscillations established in the basal ganglia are sufficient to predispose mammals to develop BEB. The Major Tasks to accomplish Specific Aim 2 are: 1) to determine whether combining synchronized basal ganglia 7 Hz oscillations with corneal irritation is sufficient to develop spasms of lid closure and other characteristics of the focal dystonia BEB; and 2) to perform control experiments to determine that theta frequency is critical in enabling the development of spasms of lid closure.

2. Keywords

Dystonia, benign essential blepharospasm, dry eye, motor plasticity, basal ganglia, deep brain stimulation, eyelids, blinking

3. Accomplishments

Major goals of the project

The overarching goal of the planned investigations is to test our hypothesis that hypersynchronized 7 Hz oscillations in the basal ganglia create a predisposing condition that transforms the normally adaptive modifications initiated by dry eye into the spasms of lid closure, excessive blinking, and trigeminal hyperexcitability characteristic of individuals with the focal dystonia benign essential blepharospasm (**BEB**). To test this hypothesis, the 1st Specific Aim of the project is to demonstrate that synchronized 7 Hz (**theta**) oscillations in the basal ganglia exaggerate plasticity in the cerebellum and excitability of trigeminal blink circuits. Major Task 1 of Specific Aim 1 is to investigate effects of synchronized basal ganglia oscillations on the activity of the deep cerebellar nucleus neurons. Major Task 2 of Specific Aim 1 is to investigate the effects of synchronized basal ganglia oscillations on the activity of superior colliculus neurons. The 2nd Specific Aim of the project is to demonstrate that synchronized theta oscillations established in the basal ganglia are sufficient to predispose mammals to develop blepharospasm. Major Task 1 of Specific Aim 2 is to determine whether combining synchronized basal ganglia theta oscillations with corneal irritation is sufficient to develop spasms of lid closure and other characteristics of the focal dystonia BEB. Major Task 2 of Specific Aim 2 is to perform control experiments to determine that theta frequency is critical for the development of spasms of lid closure.

What was accomplished under these goals?

In this year, we completed Major Task 2 of Specific Aim 2 and made significant progress on data collection on Major Task 2 of Specific Aim 2.

Specific Aim 2, Major Task 2: As described in the project narrative of the grant proposal (page 11), an important control experiment is the demonstration that the 7 Hz STN DBS is the critical frequency to produce the predisposing condition, exaggerated plasticity. As described on page 8 of the grant proposal project narrative (Trigeminal Reflex Blink Gain Paradigm), we employ our previously published paradigm that decreases trigeminal reflex blink gain in rodents as well as humans (Mao and Evinger, 2001; Ryan et al., 2014) to investigate blink plasticity. In this paradigm, high frequency stimulation of the supraorbital branch of the trigeminal nerve (**SO**) reduces the amplitude of subsequent reflex blinks, a decrease in blink gain. Our data demonstrate that 7 Hz STN DBS significantly exaggerates the reduction in blink gain relative to normal rats with no STN DBS. There is no significant difference, however, between the plasticity exhibited by normal rats without STN DBS and rats receiving 130 Hz STN DBS. In contrast 16 Hz STN DBS impairs blink reflex plasticity (Kaminer et al., 2014). If dry eye combined with deep brain stimulation of the subthalamic nucleus at 130 Hz or 16 Hz leads to spasms of lid closure, then our hypothesis that exaggerated plasticity is the predisposing condition for dystonia is false. Consistent with our hypothesis, our previous progress report provides data showing that 130 Hz STN DBS in dry eye rats does not exaggerate plasticity. The experiments in this reporting period demonstrate that combining dry with prolonged 130 Hz STN DBS or 16 Hz STN DBS does not lead to spasms of lid closure. Combining prolonged 7 Hz STN DBS with dry eye, however, produces BEB like characteristics (Evinger, 2015). These data support our hypothesis about the role of hypersynchronized 7 Hz oscillations being the basis for the predisposing condition in BEB. We are preparing a manuscript describing this work for submission. Together, the data from completing Specific Aim 2 demonstrate that the theta frequency basal ganglia hypersynchronization created by STN DBS interacts with the plastic compensatory processes initiated by dry eye to create spasms of lid closure typical of BEB.

Specific Aim 1, Major Task 2: As described in the Project Narrative of the grant proposal (page 9), the goal of these experiments is to determine how different frequencies of STN DBS affect the activity of superior colliculus neurons and how changes in collicular activity modify trigeminal reflex blink excitability measured with the paired stimulus paradigm. Predictions about superior colliculus neuronal activity alterations with STN DBS come from the basal ganglia circuit that regulates reflex blink excitability through the superior colliculus (Basso and Evinger, 1996; Basso et al., 1996; Gnadt et al., 1997). The substantia nigra pars reticulata inhibits superior colliculus neurons that excite nucleus raphe neurons. These raphe neurons inhibit spinal trigeminal blink circuits. In the paired stimulus paradigm, the first reflex blink stimulus (Condition) activates trigeminal blink circuits to evoke the Condition blink and trigeminal complex neurons also send an excitatory drive to intermediate/deep layer collicular neurons. This colliculus neuron activation provides a transient excitatory drive onto the nucleus raphe magnus neurons that inhibit spinal trigeminal blink circuits (Basso and Evinger, 1996). Thus, the second reflex blink stimulus (Test) in the paired stimulus paradigm occurs during trigeminal inhibition created by nucleus raphe magnus activation. Because of this inhibition, the Test blink is smaller than the Condition blink even though both are evoked by the same stimulus. Thus, we would predict that collicular neurons would exhibit a burst of activity after a reflex blink proportional to the Condition blink, but correlate inversely with the size of the Test blink.

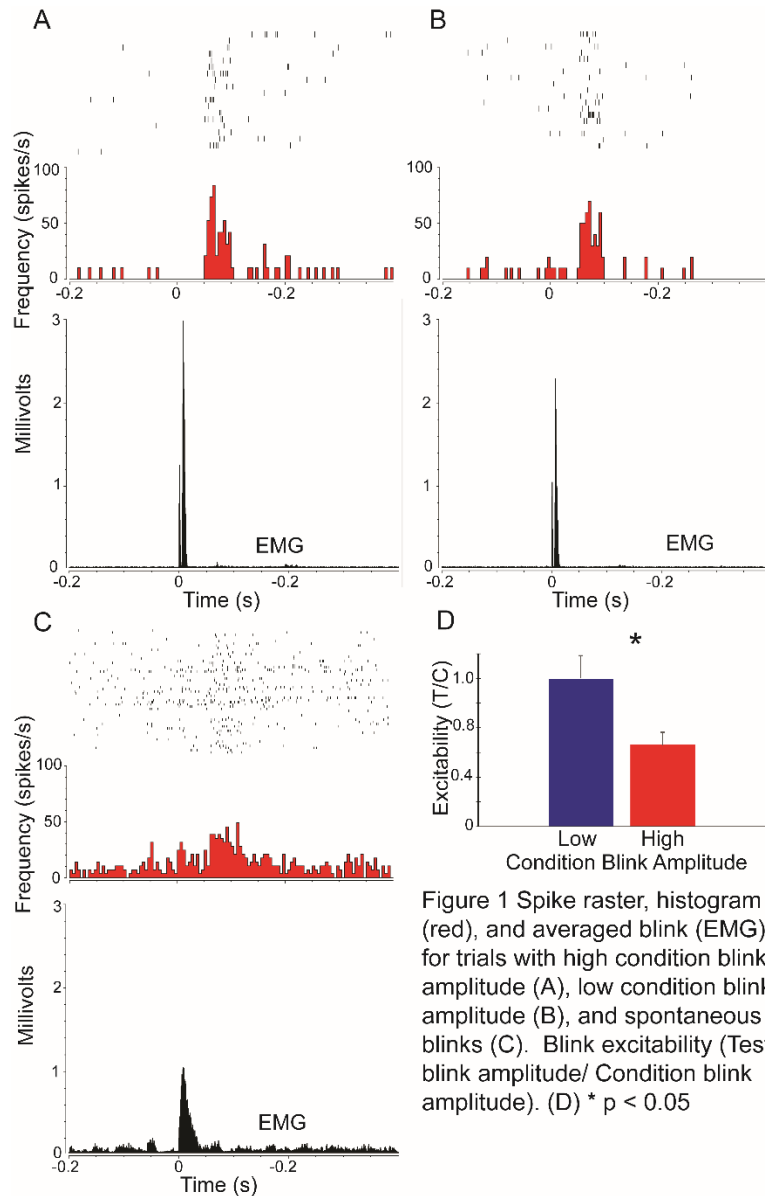


Figure 1 Spike raster, histogram (red), and averaged blink (EMG) for trials with high condition blink amplitude (A), low condition blink amplitude (B), and spontaneous blinks (C). Blink excitability (Test blink amplitude/ Condition blink amplitude). (D) * $p < 0.05$

As predicted, neurons in the intermediate and deep layers of the superior colliculus exhibit a burst of activity that begins approximately 50 ms after the Condition reflex blink stimulus (Fig. 1). For a typical neuron, the strength of this activity is proportional to Condition blink amplitude. The collicular activity following the largest one third of the Condition blink amplitudes (Fig. 1A) is larger than the collicular discharge associated with the smallest third of Condition blink amplitudes (Fig. 1B). The second component of the prediction is that the larger the collicular activity, the smaller the Test blink amplitude. We calculate the Test blink amplitude / Condition blink amplitude (Reflex Blink Excitability) for the third of the trials with high (Fig. 1A) Condition blink amplitude (Fig. 1D, red) with those with the lowest third (Fig. 1B) Condition blink amplitudes (Fig. 1D, purple). The data support our hypothesis that the magnitude of collicular activity correlates with trigeminal reflex blink excitability.

The increase in collicular discharge in reflex blinks could be due to the trigeminal blink evoking stimulus, trigeminal feedback from the lid closure, or a combination of the two sources. To identify the source of the

collicular activation, we examined collicular activity with spontaneous blinks for which the trigeminal activation of the superior colliculus comes from exclusively from feedback from the lid closure (Fig. 1C). The collicular neuron illustrated in Fig 1 C shows an increase in activity that begins approximately 50 ms after blink onset as occurs with reflex blinks (Figs 1A, B). The collicular activation with spontaneous blinks, however, is significantly smaller than that with reflex blinks. These data indicate that trigeminal feedback from lid closure is part of the trigeminal excitation and suggest that the direct trigeminal nerve stimulation to evoke the reflex blink also contributes to collicular activation

The data from the effects of STN DBS on superior colliculus activity are preliminary because the stimulus artifact from STN DBS can obscure spike activity of superior colliculus neurons. We are currently developing a software approach in MatLab to identify action potentials in the presence of stimulus artifacts. Nevertheless, the data appear to show that 16 Hz and 7 Hz STN DBS reduces the burst of activity in collicular neurons following trigeminal reflex blinks. This result can explain why both BEB and PD, trigeminal reflex blinks are hyperexcitable (Kimura, 1973; Berardelli et al., 1985; Agostino et al., 1987), *i.e.*, the amplitude of the Test blink is larger than normal.

Changes to SOW Order

There are no changes in the SOW order other than those approved in the 2016 progress report.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

I presented the data in grand round talks in the Departments of Neurology and Neurosurgery at Stony Brook University.

I published an article “Animal models are central to treating brain diseases” in the Benign Essential Blepharospasm Research Foundation patient magazine “Eyeing the Future” (Spring 2017, Vol 37, Issue 2).

We are preparing a manuscript for publication on the data from Specific Aim 2, Major Task 2.

What are the plans for the next reporting period to accomplish the goals?

The work during the next reporting period will focus on finishing the remaining work on Major Task 2 of Specific Aim 1 and completing Major Task 1 of Specific Aim 1.

Major Task 2 of Specific Aim 1 (Project Narrative page 9): The goal of these experiments is to determine how different frequencies of basal ganglia oscillations modify superior colliculus activity and to correlate these changes with alterations in trigeminal reflex blink excitability, a primary characteristic of BEB. We will record from the intermediate and deep layers of the superior colliculus receiving spinal trigeminal inputs. Our data demonstrate that both 7 and 16 Hz STN DBS increase reflex blink excitability and that 130 Hz STN DBS has no effect (Kaminer et al., 2014). We will compare the activity of superior colliculus neurons and local field potentials (LFP) with all three frequencies to the No DBS condition. Each day, rats receive 7 Hz STN DBS, 16 Hz STN DBS, 130 Hz STN DBS, and No DBS blocks of thirty trials of pairs of 2T SO stimuli with a 100 ms interstimulus interval every 20 ± 5 s. These experiments are nearing completion.

Major Task 1 of Specific Aim 2 (Project Narrative page 8-9):

The goal of these experiments is to determine how different frequencies of basal ganglia oscillations modify cerebellar interpositus (IP) activity and to correlate these changes with shifts in blink plasticity. As our data demonstrate that 7 Hz STN DBS exaggerates blink plasticity, 16 Hz STN DBS impairs blink plasticity, and 130 Hz STN DBS has no effect on the blink plasticity of normal rats (Kaminer et al., 2014), we will compare IP neural activity across all three frequencies. We will simultaneously record unitary activity and local field potentials (LFP) from blink related IP regions during SO stimulation before, during, and after our trigeminal reflex blink gain paradigm (page 8, project narrative). We will compare the activity of individual IP neurons and LFP before the trigeminal reflex blink gain paradigm to their activity after the trigeminal reflex blink gain paradigm.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

The preliminary data had an impact on the interpretation of the Neuro-Ophthalmology community as can be seen in the editorial on our manuscript in the Appendix (KB Digre, “Benign Essential Blepharospasm—There Is More to It Than Just Blinking, J. Neuro-Ophthalmol. 35(4):379-381, 2015). This article also evoked a letter Suzuki et al “Role of GABAergic System in Blepharospasm” J Neuro-Ophthalmol 2016; 36: 343-352

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. Changes/Problems

Changes in approach and reasons for change

There were no changes in approach.

Actual or anticipated problems or delays and actions or plans to resolve them

I have also been unable to attract a postdoctoral fellow or a new graduate student to replace the one who graduated. To speed up the research, I have hired a new research technician to assist with the microelectrode studies of Specific Aim 1.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

6. Products

Journal publications

Evinger C and Digre K, "Role of GABAergic System in Blepharospasm: Response" J Neuro-Ophthalmol 2016; 36: 343-352

Books or other non-periodical, one time publications

None to Report

Other publications, conference papers, and presentations

Evinger, C "Animal Models are central to treating brain diseases" Benign Essential Blepharospasm Research Foundation patient magazine "Eyeing the Future" (Spring 2017, Vol 37, Issue 2)

Website(s) or other internet site(s)

None to Report

Technologies or techniques

None to Report

Inventions, patent applications, and/or licenses

None to Report

Other Products

None to Report

7. Participants & other collaborating organizations

What individuals have worked on the project?

Name:	Leslie Craig Evinger	Cynthia Lowe	Ashley Culoso	Donna Schmidt
Project Role:	PI	Technician	Technician	Technician
Research Identifier:	0000-0002-0039-3348			
Nearest Person Month Worked:	12	12	12	6
Contribution:	Experimental design, manuscript preparation, performing experiments	Performing experiments	Performing experiments	Lab manager, histology
Funding Support:				

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

None to Report

What other organizations were involved as partners?

None to Report

8. Special reporting requirements

Not Applicable

9. Appendices

Not Applicable

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